# ADAPTIVE CHANGES IN SHADES AND COLOR OF *FUNDULUS*.

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## Contents.

## I. Introduction.

Considerable attention has been paid to the changes in shade and color which fishes frequently undergo, and the significance of the phenomenon. The subject is of some theoretical interest and has given rise to a rather extensive literature, though the conclusions arrived at by the various authors are far from unanimous.

There are two important problems involved in these investigations, namely (1) whether fishes simulate the color of the environment and (2) whether they are able to discriminate light of different wave-lengths. In other words, is the spectrum objectively speaking the same for them as for a normal human being or do they see the spectrum as a color blind person does—a gray band of varied intensity. If fishes simulate the background, this indicates discrimination of wave-lengths or color vision, for it has been established that the eye and not the skin as a whole acts as the receptor of the light stimulus. It is, of course, here not implied that they have the same color sensations as a human being has.

Several investigators have attacked the problem of color vision more directly by the method of forming food-associations with definite colors and testing fishes for their ability to distinguish these colors from others of equal brightness. Van Rynberk (1906) gives a critical survey of the earlier work on color changes in fishes and in animals generally, in which the method of food association, as well as that of instinctive response and simulation of background, have been used in testing color vision. The following brief account of the more important recent literature indicates the results obtained by investigators on color changes in various species.

Zolotnitzki (1901) using *Cheironomus* larvæ as food until fishes were accustomed to it, next presented bits of wool of the size and shape of the larvæ but of different colors. The responses to the red were more frequent than to the other colors. He concluded that they discriminate colors, but he disregarded the brightness factor.

Schöndorff (1903) maintained that the earlier workers were in error in asserting that fishes assume the color of the background, but he did so on scanty evidence.

Van Rynberk (1906) found that the flatfish, *Rhomboidichthys* simulates the environment in a striking manner.

Washburn and Bentley (1906) noted that the horned-dace, *Semotilus atromaculatus* discriminates red, green and blue.

Reighard (1908) concluded from his observations on the responses of the gray snapper, *Lutianus griseus*, that it is able to discriminate colors.

Objections have been raised to the conclusion of investigators up to this time, that there was no certainty as to brightness of light for the eyes of the fishes.

Bauer (1910 *a*) experimenting with *Charax puntazzo, Atherina hepsetus L.*, *Box salpa* and *Mugil* sp. concludes that "Wie bei normalen Menschen tritt bei Helladaption, zur Unterscheidung der Helligkeit, die Unterscheidung der Farbenwerte." He observed aversion to red or "Rotscheu" in *Charax* and *Atherina,* and Purkinje's phenomenon in *Mogil* and *Atherina.*

Hess (1910), a leading investigator in the field of color vision in animals, maintains, however, that fishes see the various parts of the spectrum exactly as a color-blind person does at any intensity of light, or as a normal person does at lowered intensity. Bauer (1910 *b*) says this is true only of dark-adapted fishes.

Von Frisch (1911, 1912, 1913) carried out many exact experiments with *Phoxinus* and other species and has come to the conclusion that fishes discriminate colors as well as simulate the background. Hess (1913) maintains, however, that the color of the background has no influence on the color of *Phoxinus*.

Freytag (1914) also found no evidence of color simulation in *Phoxinus*, but subjected the fishes to the stimulus for only twenty-four hours.

Summer (1911) found that certain flatfishes simulate the background not only in shade and color but also in pattern and his photographs given ample evidence that at least these species do so.

Mast (1916) in a very detailed study of the flounders *Paralichthys* and *Ancylopsetta,* proves that these species simulate the background in pattern as well as in shade and color and gives excellent autochromes illustrating the colors assumed.

Reeves (1919) using the method of food association and unlearned responses, and equating the light intensities, concludes that the sunfish (*Eupomotis gibbosus* L.) and horned-dace (*Semotilus atromaculatus*) discriminate light of longer wave-lengths from light of shorter wave-length and from white light, while Ohashi (1921) finds in support of Hess that goldfish and carp are unable so to discriminate, their responses being caused by different light intensities.

During the winter of 1922–23 experiments on *Fundulus heteroclitus* were carried out at the Zoölogical Laboratory, Harvard University, giving positive results which we shall now describe.

## II. Adaptive Changes in Shade.

Parker and Lanchner (1922) have recently made tests to ascertain the effect of illumination on the shade of *Fundulus*. They found that the fishes when placed in a white environment represented by a box lined with white paper and illuminated by an incandescent lamp, were of a light shade; those placed in a black environment represented by a box lined with black paper, were of a dark shade; while those placed in absolute darkness were, contrary to what one would expect, of a light shade. It was also observed that when temporarily blinded they did not exhibit any change in the various environments, showing that the action of light was not a direct one, but indirect, the eye being the receptor.

As a starting point these experiments were repeated and uniform results agreeing with these were obtained.

A test was now made to determine the effect of different colored backgrounds on the shade of *Fundulus*. Four square boxes were lined each with a differently colored paper, one side being left open for the entrance of light. The colored papers matched those named in brackets as given in Ridgway's "Color Standards and Color Nomenclature." The first was lined with light yellow paper (Lemon yellow, Pl. IV., No. 23); the second with red (Nopal red, Pl. I., No. 3, i); the third with green (Scheele's green, Pl. 6., No. 33, i); and the fourth with blue (Bradley's blue, Pl. 4, No. 51).

Fishes were first made to assume the light shade by being left for several days in a white glazed earthenware vessel in the laboratory. From this stock they were selected for experiment. Four specimens were placed in each of four battery jars filled with water to a depth of 10 cm. and the jars placed in the colored boxes. The boxes were illuminated by a white Mazda lamp (100 watts) placed at a distance of 60 cm. from the jars. For purposes of direct comparison all combinations of colored boxes taken two at a time were made, the two boxes and incandescent lamps being screened off from each other. The experiments were all carried out in a large dark room, so that the fishes in each box were under the same environmental conditions, except difference in background. The tests were each of 24-hours duration.

It was found after repeated experiments that the specimens from each colored background showed a distinct shade. When compared on a white ground in diffuse daylight, the specimens in the yellow box showed the lightest shade, those in the red box came next, the specimens in the green box were darker and in blue darkest. No change of color was noticed. All four were subject to the same light energy from source, but obviously we cannot conclude from this, that quality of light and not brightness of background, determined the effect. These tests of relatively short duration, seemed rather to indicate that brightness and not color or wave-length determined the shade. Further tests were required to settle the point.

In the next series of experiments, the effect of different monochromatic lights on fishes placed in white and black lined boxes was tried. For this purpose Wratten filters were used. These were of the larger size, namely three inches square, the smaller size which were first used not supplying sufficient light surface at the selected distance to illuminate fully the boxes. The filters selected were: Yellow (K, No. 6) Red (F, No. 29) Green (B. No. 68) and Blue (No. 45), approximately corresponding to the four colored backgrounds used in the preceding tests. The Mazda lamps were placed in boxes covered with black paper except the space occupied by the filter set in the middle of the front side. In front of the filter about 60 cm. distant, two boxes were set up, one being lined with dull white and the other with dull black paper, each being so placed as to receive equal amounts of light. All shadows were avoided.

Screened off from this apparatus, a similar one was set up in the same large dark room, so that the effect of each of two monochromatic lights on two different backgrounds could be compared under identical conditions. Each test was of 24-hours duration and comparisons were made on a white ground in diffuse daylight.

When the specimens placed in the white lined boxes were compared, they showed the same sequence in shade as in the previous set of experiments, namely from light to dark; yellow, red, green, and blue, though due to less intensity of light by use of filters, the effect was less pronounced than with the white light on variously colored backgrounds. When the effect of the same monochromatic lights on specimens in the white-lined box was compared to the effect on those in the black-lined box, it was observed that in each case the latter were darker in shade than the former. And moreover little or no difference could be observed in the degree of darkness shown by the specimens in the black-lined boxes illuminated by different monochromatic lights. The different monochromatic lights have the same effect as white light on a black background.

Parker and Lanchner (1922) suggest the hypothesis that contrast rather than simple vision is involved in responses of this kind. Keeble and Gamble (1904) state that the shade assumed by crustaceans depends on the ratio of light received by the eyes directly from the source, and that received after reflection from the background. Summer (1910) believes that this ratio holds good for fishes. Mast (1916) carried out experiments to test this hypothesis and concluded that while simulation of background is not controlled solely by light reflected from the bottom, the results of the experiments "throw considerable doubt on the hypothesis."

We have made no tests to determine whether the shade depends on this ratio. The experiments merely indicate that the background largly determines the shade. The slight differences in shade of those colored by different monochromatic light is probably due to different amounts of light passing through the filters, and that lights of all colors had a stimulating effect just as white light in causing partial or total contraction of the black pigment cells or melanophores.

From these experiments the conclusion cannot be drawn that it is quality and not quantity of light that determines the shade. The melanophores expand and contract with comparative rapidity in light of different intensities, and as no adaptive coloration was observed in these 24-hour tests the shade assumed was more probably regulated by the different intensities of the various monochromatic lights.

## III. Adaptive Changes in Color.

### *A. Effect of Colored Backgrounds.*

In order to test whether *Fundulus* simulates the background in color as some other species had been shown to do, the fishes were placed in battery jars and set in boxes lined with the same colored papers as in the first series of experiments. Here, however, the boxes with the contained fishes were placed with the open side facing the windows of the laboratory and subjected to the various color stimuli for several weeks. The water was renewed daily, and the temperature never varied more than a few degrees, remaining between about 11 and 15° C. so that the specimens were at all times under the same environmental conditions except that of color. In each jar there were 5 fishes each about 8 cm. in length. On the second days it was noticed that the shade was distinct for the fishes in each of the colored boxes, the sequence from light to darker in shade being yellow, red, green and blue. When these experiments were repeated, it was observed that these differences in shade could be recognized within three or four hours. On the sixth day there was also noticed a distinct color for each group of fishes in the four colored backgrounds. The group in the yellow box were decidedly yellow; those in the red showed a pink color; those in the green a more pronounced green than that sometimes shown by normal specimen, the region above the eyes revealing this tint in a striking manner; while those in the blue box were of a gray, slate-blue color. The adaptation in color increased during the second week when they reached their maximum degree of simulation and maintained it as long as the experiments continued, namely, for about six weeks. This was approximately the maximum time that the specimens could be kept in normal health under the artificial conditions in the laboratory and following this they gradually died. The tests were repeated many times between November 1922 and June 1923 with different sizes of fishes ranging in length from 5 to 10 centimeters and uniform results were obtained. Fundulus simulates the backgrounds after a prolonged stimulation and the effect produced cannot be due to intensity of light alone.

For purposes of comparison, fishes were also placed in three other boxes without color; one lined with dull white paper, a second with neutral gray and a third with dull black paper. Those in the white box showed the pale tint of light-adapted fish; those in the black box a very dark shade, while those in the gray showed an intermediate shade. On the other hand the fishes in the colored boxes each showed a color distinct from the others and from those in the uncolored boxes. The effect, then, cannot be due to intensity of light reflected on the background, but must be due to the color stimuli. Otherwise there is no explanation of the fact that fishes in the yellow box showed a decidedly yellow color while those in the white box which reflected all light, were pale. Although the melanophores or black pigment cells by their contraction or expansion due to intensity, indirectly influence the color to some extent by exposing or covering other color elements of the skin, the resulting effect of each colored background cannot be explained by this factor. The specific effect is due to color or wave-length.

It was first proved by Pouchet (1876) and later by von Frisch (1911) and other investigators that the expansion of the chromatophores is controlled by the sympathetic nervous system; the eyes act as receptors for the stimuli that cause changes in shade and color. For when the fish were blinded, no adaptive change in shade or color took place. In the experiments on Fundulus, collodion made opaque with lampblack was placed over the eyes and held by stitches of silk thread sewed into the superficial skin. These collodion caps generally remained on for a few days, sufficiently long to show that no adaptive change in shade takes place when fish are blinded, but not long enough to show that adaptive coloration is likewise controlled through the medium of the eye, as has been established by investigators for other species. Other methods were employed but the shock of operation caused changes in color and shade which made the specimens useless for purposes of safe comparison.

Finally it was found that making the cornea opaque with a heated needle had no perceptible influence on the healthy condition of the fishes. Specimens so treated were allowed to remain for a couple of days in order to make sure of their otherwise normal condition before being placed with other fishes in the differently colored backgrounds. In each case the blinded animal showed no simulation of the background.

It is an error to assert, as has been done by some, that blindness necessarily causes maximum darkening of the fish. It depends on the method and length of operation, and different degrees of darkening can be obtained almost at will. The possible objection that making the cornea opaque might still permit the eye to receive the stimulus and thus account for lack of darkening is met by the fact that fishes thus blinded maintain a light shade if placed in that condition on a black background, which they would not do if the eye were still functioning.

Spaeth (1913) has shown that light, temperature, and also various salts have a direct effect on the contraction of the chromatophores, both melanophores and xanthophores, when the scales with the superficial chromatophores are separated from the fish. But in our experiments with the normal fish, these factors did not influence the results, as the conditions, except background were uniform for all. Sečerov (1909) maintains that the light has a direct action, but this seems disproved by von Frisch.

### *B. Effect of Spectral Lights or Different Wave-lengths.*

The eye being the receptor of the light stimulus, the results of the above experiments can only be explained on the assumption that *Fundulus* objectively discriminates light of different wave-lengths. Since, however, intensity apparently plays an important rôle in the different shades assumed by the fish and to some extent in color, by the relative degree of expansion of the melanophores thus covering or exposing other color elements, a more rigid test was made by using spectral lights of different wave-lengths but of the same intensity.

In much of the work done on color vision in lower animals where the method of food association with definite colors was used, the factor of intensity was either totally disregarded or arrived at only approximately by comparing the relative brightness of the differently colored objects presented. This method of comparison being subjective, can give no exact quantitative data. Filters whether liquid or glass, are likewise unsafe as they are not always monochromatic, nor do they always exclude the infra-red and ultra-violet rays, and of course differ considerably in the amount of light which they allow to pass through. It is therefore necessary that either the brightness of the various spectral lights should be equated by the use of a flicker photometer, as Reeves (1919) has done in food association experiments, or the lights, varying in quality, should be made equal in the quantity of radiant energy. This latter method was first employed by Laurens (1911) at the suggestion of Prof. G. H. Parker.

More recently Laurens and Hooker (1917) have described an apparatus by means of which twenty-three lights were obtained each thirty wave-lengths in width but equal in radiant energy and extending by steps of ten from 420$\mu\mu$ to 670$\mu\mu$. The Hilger deviation spectrometer was used to obtain spectral lights and the radiant energy equated by means of a thermopile and galvanometer, thus determining the distance of the lamp from the slit of the collimator, in order to give, equal energy for all sets of lights.

This apparatus was used to test the effect of spectral lights on the color of *Fundulus*. It was necessary, however, to select only a few parts of the spectrum. In fact only two sections would be strictly necessary, one of long wave-length the other of short wave-length. Since the spectral lights are of equal energy, the fishes should show the same effect if intensity is the sole factor. If the effect varies in different spectral lights, it can be due only to wave-lengths. Using the data provided by Laurens and Hooker, the following spectral lights with corresponding conditions in the apparatus were selected.

| Range of Wave-lengths, in $\mu\mu$. | Position of Drum. | Width of Slit, Mm. | Range of Wave-lengths Either Side of *D* Line. | Distance from Lamp to Slit in Cm. |
|---|---|---|---|---|
| 470–500 | 484.0 | 3.4 | 561–622 | 4.0 |
| 520–550 | 534.0 | 2.3 | 569.5–622 | 13.0 |
| 560–590 | 574.5 | 1.8 | 573.5–606 | 19.0 |
| 620–650 | 625.0 | 1.3 | 577–601.5 | 28.5 |

For a light-source, a 1000-watt Mazda lamp was used. The lamp was placed in an iron box 12 inches square at the base and 20 inches high. On the front of the box an aperture was made for light. To secure ventilation apertures were made below in front and near the top of the rear side; these were screened by sheets of asbestos. An asbestos funnel was also placed between the aperture of the lamp box and the slit of the collimator. A metal cylinder lined with black paper and closely fitting to the ocular of the telescope conveyed the light to the jar containing the fishes, placed at a distance of 60 cm. The jar, measuring 10x5.5 cm. and 15 cm. high was covered with white paper on all sides except that exposed to the spectral light and screened off from any light leakage from lamp box. The apparatus was set up in a large dark room, so that no light other than that passing through slit of the spectroscope could enter the jar. As the light band of 30 wave-lengths is quite narrow in the red end of the spectrum, the narrow side of the jar faced the light. Four fishes about 5 cm. long were used in each test. The water was renewed by siphoning and kept at room temperature. Each test ran day and night from Monday to Saturday, when fish were examined.

In the first test, in which the fishes were exposed to the blue light waves between 470 and 500$\mu\mu$ in length, the pronounced blue color observed in specimens subjected to prolonged stimulus of blue background as noted above, was absent, the specimens not differing in any noticeable degree from some normal fish. The second test (green) was likewise inconclusive. This is the most difficult change to verify, for in light adapted fish, a green tint is always present. The third test, that of yellow—560 to 590$\mu\mu$—gave a decided response, all specimens becoming yellow. It likewise gave a basis for judging the preceding tests. For if intensity alone is a factor in the color changes, since it was equal in all the tests, the specimens in the first two should show the same yellow color as in the third. The experiment was repeated with the same fish after they had again assumed the pale tint by being placed on a white background and the response to yellow occurred again in about 36 hours. Repetition, as observed by Mast and others, increases the rapidity of the response. The fourth tests, that of red which included wave-lengths 610 to 640$\mu\mu$ also called forth a yellow response but less pronounced than in the case of yellow rays.

It is worthy of note that in all tests with spectral light of equal intensity, the shade was about the same, the melanophores being contracted. From this it may be concluded that light of any color has a stimulating effect like that of white light in causing contraction of the melanophores. It shows clearly that intensity of light plays an important rôle in the expansion or contraction of the melanophores and thus indirectly affects the colors of fishes by exposing or obscuring other color elements. Nevertheless, the fact that the yellow part of the spectrum caused a decisive change to a yellow tint by expanding the xanthophores or yellow pigment cells, while the intensity remained the same as in other spectral lights to which the fishes were exposed, is definite proof that in *Fundulus,* the quality or wave-length has a specific effect apart from quantity of light or intensity. That the responses to the various colors is here not so pronounced as in experiments where fishes were placed in colored boxes and exposed to diffuse daylight for several weeks, is due to the fact that the fishes were exposed to spectral lights for only five day periods. Moreover, the area and brightness of the colored environment were much reduced.

## IV. The Basis of Adaptive Changes in Shade and Color.

### *A. The Normal Coloration.*

The specimens freshly taken from the sea varied greatly from a yellowish green to black when seen from above. The silvery bars of the adult male with scattered yellow and bronze colored spots are very conspicuous on specimens otherwise dark. The bars are due to the smaller number of melanophores superficial to the scales and to the presence of dense groups of guanophores or iridocytes, which are frequently arranged like branched filaments. The guanophores are highly refractive and when viewed by reflected light, reveal the whole gamut of colors.

As previously stated all specimens were made to assume the pale yellow green tint by being placed for several days in a white dish before being subjected to the various other colored environments. If, now, the living specimens be examined by reflected light under a microscope or with a good hand-lens, it will be seen that there is more than enough material to work on to account for the colorations revealed in the various experiments. The most striking phenomenon is the presence of brilliant points scattered over the dorsal and lateral surfaces. This is especially prominent when direct sunlight is allowed to fall on the fish. The bright points occupy the central portion of many melanophores and in their dark setting appear like jewels. The phenomonon in due to an association of iridescent guanophores or iridocytes with melanophores.

For further detailed study of the color elements, fresh specimens from each of the colored environments were fixed by dipping them in hot water for about 10 seconds, then in cold and finally examining them in glycerine. When examined under higher magnification, the guanophores are seen to be spindle-shaped bodies having an average length of about $20\mu$ and a maximum breadth of 3 to $4\mu$. There are other smaller guanophores or iridocytes, ovoid in shape being about $11\mu$ in length and 3 to $4\mu$ in width. These iridocytes are especially found in patches apart from any association with melanophores and give rise to the mottled condition of the male. When associated with melanophores, the guanophores seem to acquire a more brilliant iridescence than when they occur separately. The more common colors reflected in the specimens adapted to a light background are yellow, orange, and green. If the scales are removed, and examined under the microscope, these iridescent points are not to be seen in the melanophores, which remain on the distal portion of the scales after removal. They are located on the proximal portion of the scale and the reflected rays pass through the distal portion of the overlapping scale.

It is well known that many animals show a green coloration though there is no green pigment present. This is true of *Fundulus*. The green tint is due to the combination of the effects of melanophores and xanthophores over a layer which reflects blue or over iridocytes possessing that physical property which Pouchet termed "le cerulescence." When paraffin sections are made through the skin there is to be seen a compact layer of cells occupying the inner part of the dermis next to the muscles. The cells are rectangular in shape with their long axis parallel to the surface. The nuclei are likewise elongate. Cunningham and McMunn (1893) have suggested an ontogenetic relation existing between this layer which they term the argenteum, and the iridocytes.

There is also a close relation in structure and optical properties between the guanophores of the integument and those of the peritoneum. The peritoneum of *Fundulus* is covered with dense black pigment on the surface exposed when the body cavity is opened, and the inner surface shows the typical silvery sheen. This layer is more highly specialized than the reflecting layer of the integument. It does not stain with anilin dyes. If the pigmented surface be examined by deflected light, the same phenomenon of brilliant points associated with melanophores is seen, showing that the guanophores have the same optical property as was present in the integument.

### *B. Shade Adapted to White, Gray, and Black Backgrounds.*

As is well known for many species, changes in shade are due to contraction or expansion of the melanophores. Ballowitz (1893) has shown that the chromatophores of fishes are innervated by branches which proceed from a dense nerve-net surrounding the chromatophores, or even direct from a nerve bundle itself. These constitute the true pigmento-motor nerves. According to Ballowitz, they have many free terminations on and within the chromatophores. As already pointed out, the eye acts as the receptor and the stimulus is conveyed by way of the sympathetic nervous system to motor fibers innervating the chromatophores as Pouchet surmized and von Frisch clearly demonstrated. Von Frisch (1912) maintains that a centre for contraction of the melanophores exists at the anterior end of the medulla.

On a white background the melanophores and xanthophores are contracted. On a black background the melanophores are expanded and the xanthophores are contracted. In the male specimens the silvery bars and the bright mottled condition of the lateral portions are retained no matter how long the fishes are left in this environment. In fact, they are more pronounced by contrast with the dark ground-color of the fish. No evidence of a reduction or increase in the number of melanophores after a prolonged stimulus in the white or black environment was observed, such as Kuntz (1917) asserts to be the case in *Paralichthys*. On a gray background the specimens were intermediate in shade, the melanophores being partially contracted.

### *C. Yellow Adapted.*

The melanophores in specimens exposed to a yellow background for several weeks were maximally contracted. The xanthophores on the other hand were maximally expanded, to such an extent that they appeared diffused and the limits of the individually pigment cells were difficult to determine. In this condition, the blue reflecting layer is largely concealed. Examined by a reflected light, the brilliant points on the scattered melanophores are very striking, the predominant colors reflected being yellow and orange.

### *D. Red Adapted.—Vaso-dilation.*

The melanophores on the superficial distal portions of the scales were likewise contracted in fishes exposed to a red environment for the same period as in the preceding test, but those in the proximal portions and in deeper parts were not fully contracted. The xanthophores, however, were not fully expanded as in the yellow adapted fish. There was a denser spherical concentration of the pigment giving an orange tint. There are no red pigment cells in *Fundulus*. But the color of the specimens adapted to a red background, was quite distinct from that in the yellow. Instead of the bright yellow of the latter they showed a pink color. It was at first thought that this might be caused by the partially contracted xanthophores giving a deeper yellow or orange to the cells, in combination with the melanophores. On further examination it was seen that the dorsal region of the head, usually pale, was quite red due to the dilated condition of the blood capillaries. The opercular region showed a striking network of vessels. The capillaries of the trunk were likewise dilated giving a pink color to the fish. Before the melanophores are more fully contracted by the prolonged stimulus, the color is brownish.

The pink color is without doubt, due to the blood in the capillaries. As in the cases of experiment with fishes on other colored backgrounds which ran concurrently through the winter months, the results here were always the same. The only exceptions were those fishes taken late in the spring which did not seem normal and were affected by the sudden change in temperature on being taken from the sea. Many of these died and those that lived were exceedingly slow in assuming the light shade when placed in a white vessel before being exposed to the red environment. And strangely enough, though not revealing any blood vessels over trunk and head, the caudal find of one of these specimens was very red, due to vaso-dilation. The general coloration of these late-spring specimens on a red background approached the yellow, but was more sombre. The fact that in these specimens lacking the pink coloration, the blood capillaries were not visible, as in other specimens on a red background, confirms this explanation.

While the distinctive color is then due to the blood in the capillaries, there still remained the possibility that the cutaneous vessels were equally dilated in fishes adapted to other environments, but that the vessels were covered with melanophores. This seemed to be true of the blue adapted fish, for the dorsal region showed dark lines as if the melanophores were especially abundant and expanded over the capillaries. But the anterior portion of the head, where few melanophores are present, failed to show any dilation. Again, the fishes adapted to a yellow background where melanophores are maximally contracted and likewise those adapted to a white background where both melanophores and xanthophores are contracted, showed no evidence of vaso-dilation.

### *E. Green Adapted.*

In those adapted to the green environment, the shade was darker than in the preceding cases, due to partial expansion of the melanophores. The xanthophores were only slightly expanded. The most striking portion of the body in which this color was emphasized, was above the eyes.

### *F. Blue Adapted.*

In these specimens the melanophores lying in the deeper portions of the integument, and those lying upon the proximal portion of the scales were expanded, thus giving a grayish tone when seen through the overlying adjacent scales. On the distal portion of the scales, the melanophores were generally contracted. The xanthophores were maximally contracted thus exposing the blue reflecting layer. When the surface of the skin was examined by reflected light, the predominant color coming from the brilliant points was a bluish green.

## V. DISCUSSION

From the brief outline at the beginning of this paper of the more recent work on the problem of simulation of fishes to the background, it is evident that the majority of investigators have found that the various species tested, do simulate the background and discriminate light of different wave-lengths. To these species, *Fundulus* can unquestionably be added.

Mast (1916) who made a detailed study of two species of flounders and observations on other fishes kept in the aquaria at

Beauford, comes to the general conclusion that "adaptive changes in shade occur in the skin of practically all of the different fishes in the region of Beaufort, N. C.; adaptive changes in color in many, but adaptive changes in pattern in only few."

When the problem of color discrimination is attacked directly by the method of unlearned responses or by forming food associations, the factor of light intensity must be the same for all wave-lengths. Hess, who has done most in the field of color responses in animals generally, stoutly denies that conclusive proof has been brought forth for color vision in fishes, and Parsons (1915) accepts Hess's conclusion.

One benefit of this criticism has been to bring about the use of more precise method in testing the responses of fishes to different colors. Reeves (1919), however, equated brightness for different colors and concluded that fishes discriminate light of different wave-lengths. On the contrary, Ohashi (1921) concludes that intensity alone is responsible for the different responses in the goldfish and carp when subjected to monochromatic light. As this is the most recent paper that has come to our notice, and as the author arrives at conclusions different from those stated in the present paper, we shall discuss Ohashi's work in detail.

Ohashi first noticed that goldfish up to three years old were attracted to different colored lights when subjected in turn to these stimuli. In other words they are positively phototropic to all monochromatic lights.

In the following experiments red and green liquid filters were set in the top of an aquarium equidistant from the ends and the lamp placed midway between them. The fish assembled under the green light which was the brightest for them. When the lamp was adjusted so that the green and red appeared of equal brightness to the observer, the fishes assembled in about equal numbers under each color. From these observations Ohashi concludes that no support for the belief that fishes have color vision can be found here. Apart from the fact that the liquid filters admittedly did not give pure monochromatic lights, there is no certainty that the apparent equality of brightness of the two lights to the human eye were likewise of equal brightness to the eyes of the fish. In short, brightness cannot be objectively determined by simply comparing the different colored lights and no valid conclusions can be drawn from tests of this kind.

In his food association experiments, Ohashi noted that fishes could not discriminate red when this color was plainly visible to the human eye. This does not disprove color discrimination in fishes for it is quite probable that at a lowered intensity they would be unable to discriminate color and yet be able to do so above a certain threshold of intensity. Hess, in fact, has shown that the red end of the spectrum is shortened for fishes.

When subjected to prolonged stimulation by red and by violet lights the fish became reddish yellow and bluish and on examination it was found that the melanophores expanded in blue light and the xanthophores in yellow light. Unless, therefore, there is a direct effect of light on the chromatophores as Šecerov claimed, but which von Frisch and Mast deny, the reported adaptive coloration must be due to color discrimination.

Ohashi next placed fishes on eight bottoms of colored paper; blue (two tints), red (also two tints) white, black, and gray (two shades). Changes in color were noted in 30 seconds and completed in two minutes, the principle colors noted being dark, reddish yellow and blue. If we have here a case of adaptive coloration, it would indicate a remarkable rapidity in the response. Obviously it is only adaptation in shade, as the author finally concluded when all specimens were compared with one another and it was noted that those which had been on a dark red bottom were similar in shade to those on a grey. The period was entirely too short, however, to conclude from this experiment that they are unable to simulate the background in color.

The same may be said regarding his final experiments where fishes were subjected to the stimulus of red and blue from filters, those in the red becoming reddish yellow and those in the blue, dark within five minutes. When the blue light was made more intense than the red, the fishes in the blue light became yellow and those in the red dark. Again intensity of light was no doubt the factor causing different degrees of contraction of the melanophores as the author concludes and as we have noted for *Fundulus*. This however, does not disprove color discrimination for these species. On the contrary, the results following prolonged stimulus of different colored backgrounds indicate that the species tested do simulate the background.

The subjective significance of the phenomonon of color simulation. is not considered here. It is important first to know, as Uhlenhuth (1911) pointed out, whether lights of different wave-length bring about different responses. This has been found to be true of Fundulus heteroclitus.

This research was suggested to me by Prof. G. H. Parker and carried out under his supervision. To Prof. Parker I am indebted for many helpful suggestions, for the privileges of the Harvard Laboratory and for many other courtesies extended while making this investigation.

## VI. Summary

1. Adaptive changes in shade occur in *Fundulus heteroclitus* when placed on white, gray, and black backgrounds. This adaptation is brought about by the contraction or expansion of the melanophores.

2. On colored backgrounds for short periods, adaptation in only shade occurs.

3. Prolonged stimulation by colored backgrounds brings about adaptation in color. This occurred in yellow, red, green, and blue environments.

4. The experiments with spectral lights of different wave-lengths but of the same radiant energy or intensity, show that the melanophores respond similarly at all wave-lengths, the contraction being due to light intensity.

5. The response to the longer wave-lengths, red and especially yellow, in causing an expansion of the xanthophores, though intensity was the same as in the blue or short wave-length end of the spectrum, shows that the quality of light or wave-length has a specific influence on the coloration of *Fundulus*.

6. The stimuli causing changes in shade and color are received through the eyes.

7. The changes in color are brought about by the degree of expansion or contraction of the melanophores and xanthophores combined with the optical properties of the guanophores or irido-

cytes and the reflecting layer. The guanophores are frequently found associated with the melanophores.

8. Adaptive change to a yellow background is brought about by a maximum expansion of xanthophores and a maximum contraction of the melanophores.

9. In fishes adapted to a red background, the xanthophores are only partially expanded and the melanophores contracted. The pink color is due chiefly to a dilation of the blood capillaries.

10. In fishes adapted to a green background, the green tints present in the normal light adapted fish, is increased and is especially noticeable above the eyes. The xanthophores and melanophores are partially expanded.

11. In blue adapted fishes the xanthophores are maximally contracted and the melanophores in the deeper portions of the dermis and upon the proximal parts of the scales, are expanded.

*Postscript.* Since the preparation of this paper I have had the opportunity of repeating the experiments on *Fundulus* at the St. Andrew's Biological Station. My results have been the same as those obtained in the Harvard Laboratory except that I failed to get on a red background the pink coloration and vaso-dilation described in this paper. Whether this is a seasonal or a local difference in the fishes remains to be worked out.

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